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SYMBIOTIC ASSIMILATION OF CO_2 IN TWO HYDROTHERMAL VENT ANIMALS, THE MUSSEL *BATHYMODIOLUS THERMOPHILUS* AND THE TUBE WORM *RIFTIA PACHYPTILA*[1]

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Abstract

Shipboard studies showed major differences in the incorporation of CO_2 by the specific prokaryotic symbionts of two deep-sea vent invertebrates. The rate of CO_2 fixation was optimal at approximately 22°C in fresh trophosome material from the pogonophoran tube worm *Riftia pachyptila.* Sulfide, but not thiosulfate, served as the electron donor. Thirty-five percent of the aerobic rate remained in deoxygenated samples presumably due to traces of hemoglobin-bound oxygen. Gill preparations from the mytilid mussel *Bathymodiolus thermophilus,* however, assimilated CO_2 with a maximum rate at 12° to 15°C. Thiosulfate, but not sulfide, served as the electron donor. The activity was completely inhibited in deoxygenated samples. These metabolic dissimilarities between the symbionts of the two hosts extend to DNA base ratios and cell sizes. Partial fractionation of the mussel gill preparation yielded a prokaryotic or "bacterial" fraction that showed a CO_2 assimilation rate three-fold higher than that of the crude homogenate. This activity was not affected by the *in situ* hydrostatic pressure of 250 atm (100% barotolerance). The mussel gill symbiont represents the first sulfur-oxidizing vent prokaryote with psychrophilic growth characteristics. The natural distribution of *R. pachyptila* and *B. thermophilus* within the physico-chemical regime of the immediate vent vicinity corresponds well with the metabolic differences of their symbionts.

Introduction

The extensive animal populations found at hydrothermal vents along the East Pacific Rise fracture zone were proposed to be supported by chemosynthetically produced organic carbon (Lonsdale, 1977; Corliss *et al.,* 1979; Jannasch and Wirsen, 1979). Indeed, during the first biological studies, the mixing zone of the highly reduced hydrothermal fluid with the ambient, oxygen-containing seawater was found to support large numbers of chemoautotrophic bacteria (Jannasch and Wirsen, 1979, Karl *et al.* 1980).

From their unusual abundance it was apparent that certain vent animals derived their food supply by means other than filtration of suspended bacterial cells. The symbiotic nature of their nutrition was soon proved by morphological and/or enzym-

We dedicate this paper to Professor Norbert Pfennig, University of Konstanz, in honor of his 60th birthday.

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ological studies (Cavanaugh *et al.,* 1981; Felbeck, 1981; Felbeck *et al.,* 1981; Cavanaugh 1983). While the giant white clam, *Calyptogena magnifica* (Boss and Turner, 1980), and the mytilid mussel, *Bathymodiolus thermophilus* (Kenk and Wilson, 1985), appear to harbor prokaryotic cells within their gill tissue, these cells are concentrated as a separate tissue, the trophosome, within the coelomic cavity of the mouth- and gutless pogonophoran tube worm *Riftia pachyptila* (Jones, 1981).

These three invertebrates are the most conspicuous of the vent animals, but their relative abundances differ from vent to vent. *Calyptogena* and *Riftia* dominated at the "21°N" site (East Pacific Rise) with the mussel being absent. By contrast, clusters of tube worms and mussels composed most of the biomass in other vent sites (Grassle, 1985). Of these locations, the highest standing animal crop was reported at the site known as "Rose Garden" in the Galapagos vent system (Hessler and Smithey, 1983). This site was revisited in March 1985.

During the latter expedition we studied the assimilation of ^{14}C-labeled bicarbonate by homogenized *Riftia* trophosome and *Bathymodiolus* gill tissue focussing on the effects of temperature, oxygen, pressure, and the type of sulfur compound used as the energy source.

Materials and Methods

Collection and storage of specimens

During the March 1985 Galapagos expedition, live animals were collected at a depth of 2550 m at the "Rose Garden" vent site (0°84.25'N, 86°13.48'W) on 14 out of 18 DSRV ALVIN dives. Upon collection, the animals were placed in a closed insulated container designed to keep the contents at deep sea temperature (*ca.* 3°C) during the submersible's ascent. A minimum of 2 h elapsed between collection of animals and their availability on the surface. Immediately upon return to the surface, the animals were removed, placed on ice in a 4°C refrigerator, and dissected for the experiments within one hour. Only live animals, as evidenced by the tight closure of the shells (*Bathymodiolus*), or by the characteristic withdrawal into the tube (*Riftia*), were used.

Medium

For all experiments, an artificial seawater medium (ASW), as described by Ruby and Jannasch (1982), was used with the concentration of $NaHCO_3$ raised to 2.5 m*M* and the Tris omitted. A phosphate buffer (3.0 mmolar) was sufficient to keep the pH constant at 7.5 at all experimental conditions.

Homogenate preparation

Mussel gills were excised from the animal taking care not to include fragments from other tissues. Prior to homogenization, the gills were rinsed three times with 40 ml sterile seawater. Trophosome tissue, which was too loose in texture to be similarly washed, was removed aseptically with minimal amounts of worm tissues present.

These tissue samples, approximately 5 ml in volume, were mixed with equal volumes of 4°C sterile ASW and gently homogenized in an ice-cooled ground glass homogenizer. The protein concentration of the mussel gill preparation ranged from 18 to 27 $mg \cdot ml^{-1}$, and that of the worm trophosome between 60 to 97 mg protein $\cdot ml^{-1}$. The homogenate was kept on ice until its dilution into the reaction medium, usually for not more than 10 min.

Experiments

The concentrated homogenates were diluted 100-fold in 4°C ASW and distributed in 15 to 20 ml aliquots to 50 ml rubber-stoppered flasks. After preincubation at selected temperatures for 10 to 20 min, $NaH^{14}CO_3$ (50 μCi · ml^{-1}) was added in various amounts to yield final specific activities of 0.05 to 0.10 mCi · $mmol^{-1}$. At appropriate intervals, 2 to 3 ml samples of the suspension were filtered through 25 mm GF/F filters (Whatman), and rinsed with 3 volumes of 4°C sterile seawater. The filters were then fumed for 20 min over concentrated HCl and dried in air. The radioactivity incorporated into the cells was measured with a Beckman liquid scintillation counter (Model LS 100C).

To achieve O_2-free conditions, ASW, prior to $NaHCO_3$ addition, was bubbled with O_2-free N_2 gas for one hour. The homogenate and $NaHCO_3$ (labeled as well as unlabeled) were then added, and 15 ml aliquots were distributed to 17 ml Hungate test tubes (Bellco Glass, Inc.). All the above steps were carried out on ice under a nitrogen gas flow. A 20 ml portion was transferred to a flask and aerated to serve as an aerobic control.

To measure the effect of pressure, 3.5 ml serum stoppered glass tubes were completely filled with well aerated, pre-mixed reaction medium. The tubes were then either left at 1 atm or placed into stainless steel pressure cylinders which were immediately sealed and pressurized to 250 atm. The time between isotope addition and attaining full pressure was approximately 5 min. At each point, samples were treated as described above.

Fractionation of mussel gill homogenate

Five fractions of mussel gill homogenate were prepared as follows: The crude homogenate was centrifuged for 5 min at low speed (3000 × *g*) in a table-top centrifuge. The pellet was resuspended in ASW medium and recentrifuged as before. The two supernatants were combined, and centrifuged for 6 min in an Eppendorf microfuge (Model 5414) at 15,000 × *g*. The resulting five fractions were: the crude homogenate, the low and high speed supernatants, and the pellets of the low and high speed centrifugations. The last of these was designated the "purified" bacterial fraction.

Protein determination

Aliquots of tissue homogenates were supplemented with trichloroacetic acid (10% w/v final concentration) and protein was precipitated by heating (90°C, 20 min). After pelleting at room temperature (15,000 × *g*, 10 min) protein was dissolved in 0.1 *M* NaOH (50°C, 30 min) with several gentle mixings. It was then measured by the Coomassie Brilliant Blue dye binding technique of Bradford (1976) using a microassay and commercially available dye reagent as previously described (Nelson *et al.*, 1982).

Sulfide preparation and determination

A 200 m*M* stock solution of Na_2S was prepared after the method of Brock *et al.* (1971) and autoclaved. A volume was neutralized with HCl just before use and added to experimental flasks at 100 to 1200 μM final concentration. At the conclusion of the cruise, the stock solution still contained 93% of its initial sulfide concentration as determined by the method of Cline (1969). The sulfide concentrations stated for our experiments are only valid for the start of the experiment as sulfide at neutral pH in aerobic seawater has a half-life of approximately 1 h (Almgren and Hagström, 1974).

DNA

DNA was extracted from homogenized gill tissue (2.5 g wet wt) and from the "purified" bacterial fraction (0.75 g wet wt). It was deproteinized, purified by repeated washings after binding to hydroxylapatite, and dialyzed. These procedures, as well as methods for estimating base composition, genome size, and relative proportion of more rapidly renaturing DNA, were as described previously (Nelson *et al.,* 1984).

Results

Electron donor

Figure 1 represents the data of experiments testing the capacity of two sulfur compounds, Na_2S and $Na_2S_2O_3$, to support CO_2 incorporation in mussel gill and in worm trophosome homogenates. It appears that the *Bathymodiolus* gill tissue can utilize thiosulfate but not sulfide while the pattern is reversed in the *Riftia* trophosome tissue. In both cases poisoned samples (1.5% glutaraldehyde) and homogenized host tissues (mussel mantle or worm vestimentum) showed CO_2 incorporation which did not increase beyond the "zero time" level. For *Bathymodiolus* (data not shown) these values were less than 0.5 nmol CO_2 mg^{-1} protein in assay mixtures supplemented with 400 μM $Na_2S_2O_3$.

While the enhancing effects of thiosulfate and sulfide were reproducible, a certain degree of variability was observed in both cases in the activity of the unsupplemented preparations. This correlated with the length of the preincubation period which preceded the introduction of the radioactive substrate. The results in Figure 1 were obtained after approximately 15 min of preincubation. When this period was increased to 75 min, no activity was detected in the mussel preparations without thiosulfate.

In addition to electron donor specificity, other differences between the two active preparations were in the reaction's duration and linearity. Whereas the activity of mussel gill tissue was linear for 2 to 3 h or more, that of the *Riftia* trophosome prep-

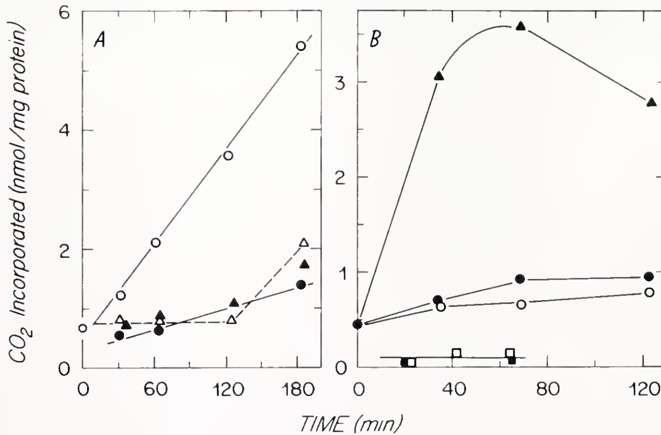

FIGURE 1. Incorporation of CO_2 in *Bathymodiolus* gill (A) and *Riftia* trophosome (B) homogenates in the presence of two electron donors. A) (○) 400 μM $Na_2S_2O_3$; (▲) 100 μM Na_2S; (△) 500 μM Na_2S; (●) no additions (tests were done at 6°C and the diluted homogenate contained 240 μg protein ml^{-1}). B) (○) 500 μM $Na_2S_2O_3$; (▲) 600 μM Na_2S; (■) 600 μM Na_2S plus 1.5% glutaraldehyde; (●) no additions (tests were done at 12°C and the diluted homogenate contained 600 μg protein ml^{-1}); (□) diluted vestimentum homogenate (850 μg protein ml^{-1}) plus 600 μM Na_2S.

aration was linear for less than 1 h. The incorporated $^{14}CO_2$ actually decreased after that time period (Fig. 1B). This phenomenon, which may indicate cell lysis or leakage of labeled solutes, could not be prevented or postponed by the addition of fresh sulfide after 4 min, the approximate time at which no more sulfide could be detected in the medium.

Various concentrations of the two electron donors were tested, and the results are presented in Figure 2. The mussel gill homogenate was equally active with all thiosulfate concentrations tested (100 μM or higher), although it was not determined whether higher concentrations may support the activity for longer periods of time. The optimal sulfide concentration for the *Riftia* trophosome was approximately 600 μM. At 1.2 mM sulfide the rate of CO_2 incorporation was about 40% of the optimal, but was relatively short in duration (20 min).

Temperature

The data of Figure 3 show a distinct difference in the temperature range favored by the two crude preparations. The "purified" fraction of mussel gill preparation behaved very similarly, if not identically, to the crude homogenate. Optimal temperature for CO_2 incorporation was 15°C or lower (four separate experiments) for the mussel and approximately 22°C for the worm. At 0°C the worm preparation exhibited about 5% of maximal activity while the mussel preparation was significantly more active. In four temperature profiles for *Bathymodiolus* homogenate, the CO_2 incorporation at 0°C averaged 42% (±17%) of the maximum. At the other end of the temperature range tested, the trophosome preparation was still active at 28° to 30°C, whereas the mussel preparation became inactive at approximately 20°C.

Oxygen

In view of the different redox potentials conferred by the two sulfur compounds serving as electron donors, the rate of CO_2 incorporation was tested for its response to the availability of O_2, the oxidizing agent commonly used by chemoautotrophic bacteria. As demonstrated in Figure 4A, the crude mussel gill preparation showed no

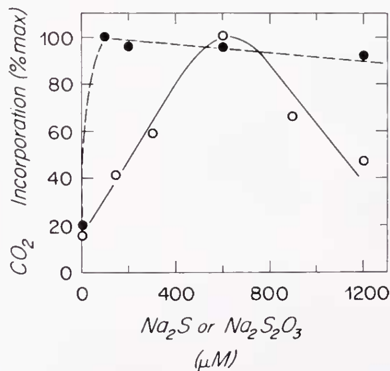

FIGURE 2. Incorporation of CO_2 in *Bathymodiolus* gill (●) and *Riftia* trophosome (○) homogenates at various concentrations of thiosulfate and sulfide respectively (tests were done at 6° and 12°C and the diluted homogenates contained 240 and 720 μg protein ml^{-1}, respectively). Maximum CO_2 incorporation rates in these experiments were 1.7 and 2.0 nmol mg^{-1} protein h^{-1}, respectively.

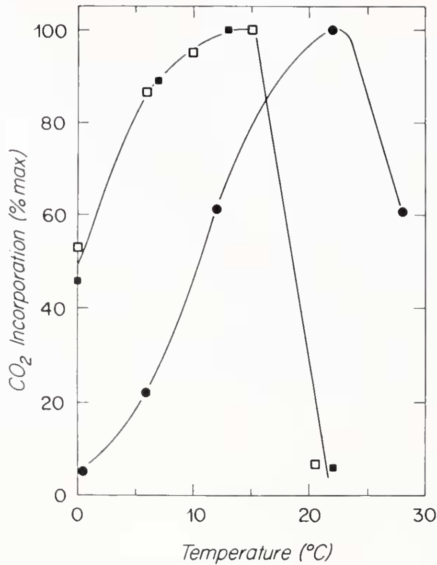

FIGURE 3. Incorporation of CO_2 in *Bathymodiolus* gill and *Riftia* trophosome homogenates at various temperatures; (□) mussel gill homogenate incubated with 200 μM $Na_2S_2O_3$; (■) "purified fraction" of mussel gill homogenate incubated with 400 μM $Na_2S_2O_3$; (●) trophosome homogenate with 600 μM Na_2S. Maximal rates in these experiments were 6.0, 1.0, and 3.3 nmol CO_2 mg^{-1} protein h^{-1}, respectively.

activity under anaerobic conditions. The activity of the *Riftia* preparation also was reduced, but only by approximately two-thirds (Fig. 4B). Here, differences in incorporation between duplicate "anaerobic" preparations (at or before arrow) may reflect differences in the amount or availability of oxygen bound to traces of *Riftia* blood. The short-term stimulating effect of aeration appears to be significant in restoring CO_2 incorporation to its initial rate for a period of approximately 20 min.

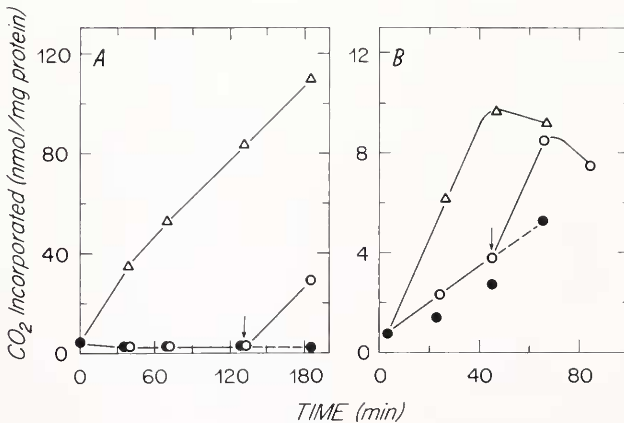

FIGURE 4. Incorporation of CO_2 in *Bathymodiolus* gill "purified" preparation (A, 320 μg protein ml^{-1}, incubated at 8°C with 400 μM $Na_2S_2O_3$) and *Riftia* trophosome homogenate, (B, 970 μg protein ml^{-1} incubated at 14°C with 600 μM Na_2S) under (●) N_2, (○) N_2, air introduced at arrow, and (△) air.

"Purified" Bathymodiolus *gill homogenate*

Since the *Riftia* preparation proved to be very unstable, we concentrated our purification efforts on the mussel gill homogenates. The activities measured with the "purified" preparation (see Materials and Methods) were very similar to those of the crude homogenate with respect to their response to temperature (Fig. 3) and to the utilization of thiosulfate as the electron donor (Fig. 4). The specific activity of the "purified" fraction was up to 3-fold higher than those of the crude homogenates (30.2 *vs.* 9.4 nmol CO_2 mg^{-1} protein h^{-1}).

Appropriate dilutions of all five fractions (see Materials and Methods) were assayed for CO_2 incorporation, measured for protein, and also stained (acridine orange) for examination using epifluorescence microscopy (Hobbie *et al.,* 1977). This examination revealed highly stained spheres or short rods of uniform appearance having a diameter of 0.5 to 0.75 μm. It was calculated that there were approximately 5×10^{10} of these cells per cm^3 of intact gill tissue. When CO_2 incorporation of each of the five fractions was normalized to the number of these cells present, values within the narrow range of $0.7\text{–}1.8 \times 10^{-8}$ nmol CO_2 $cell^{-1}h^{-1}$ were obtained. On the other hand, CO_2 incorporation expressed per unit protein varied by over 200 fold (0.1–23 nmol CO_2 mg^{-1} protein h^{-1}) for the same five fractions.

Pressure

The CO_2 incorporation by the "purified" fraction of the mussel gill homogenate was measured at the *in situ* pressure (250 atm) of the animal's habitat and compared to a 1 atm control. The data of Figure 5 show that, at least over the 1 h period, the applied pressure appears to have no significant effect on the measured activity.

DNA

The host DNA in the mussel gill has a base ratio of approximately 28 to 33 mol % guanine + cytosine (% G + C). The symbiont DNA has approximately 34 to 38% G + C and a genome size within 20% of that of *E. coli.* These assignments are based on the observation that the higher G + C content DNA became more abundant in the "purified" fraction with a parallel increase in the fraction of DNA possessing faster

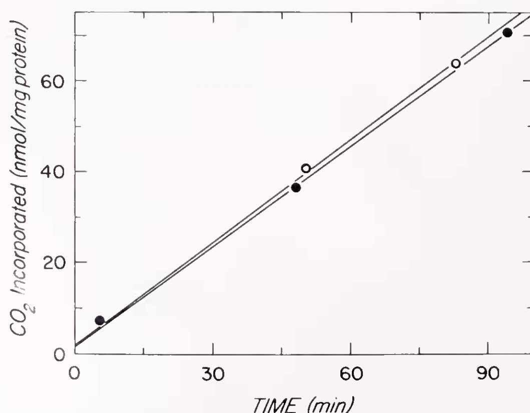

FIGURE 5. Incorporation of CO_2 in the "purified" fraction of *Bathymodiolus* gill homogenate (380 μg protein ml^{-1}) at 9°C with 400 μM $Na_2S_2O_3$, (●) at 1 atm and (O) at 250 atm.

TABLE I

Characterization of DNA of hosts and symbionts by the method of thermal denaturation and renaturation

Name	Host	Symbiont	
	Base ratio (mol % G + C)	Base ratio (mol % G + C)	Genome size[a] ($\times 10^9$ dalton)
Riftia pachyptila[b]	34–44	57–59	2.1–2.2
Bathymodiolus thermophilus[c]	28–33	34–38	1.9–2.0

[a] *Escherichia coli*, strain 620 K-12F (genome = 2.4×10^9 dalton), used as standard.
[b] From Nelson *et al.*, 1984.
[c] Based on DNA extracted from (1) crude homogenate, *B. thermophilus*, (2) "purified" friction, *B. thermophilus*, and (3) *E. coli* standard. Genome size calculated assuming that 90% of DNA of "purified" fraction was from symbiont.

(non-eukaryotic) renaturation kinetics. Both of these changes paralleled the higher CO_2 fixing capacity of the "purified" fraction. These data are presented (Table I) along with corresponding data for *Riftia* and its symbiont. Of the DNA obtained from homogenized trophosome tissue, approximately 90% was that of the symbiont (Nelson *et al.*, 1984). By contrast, symbiont DNA comprised only about one-third of the DNA obtained from the crude homogenate of *Bathymodiolus*.

DISCUSSION

In view of the absence of symbiont isolates, the data reported in this paper are hoped to help in elucidating the types and the mechanisms of the symbiotic systems in *Bathymodiolus thermophilus* and *Riftia pachyptila*. In both cases the CO_2-fixing tissue (gill or trophosome) contained a significant fraction of prokaryotic DNA as indicated by their genome sizes (Table I). The latter were well within the range for those of free living prokaryotes (1 to 3×10^9 dalton) and considerably greater than those of obligate symbionts (Soldo *et al.*, 1983). While sufficiently large genome sizes do not guarantee that either symbiont may eventually be grown free of its host, they imply that the present lack of success cannot be ascribed to a major loss of genetic information.

The two symbionts also differed in their DNA base compositions, the *Riftia* symbiont falling within the range characteristic of the genus *Thiobacillus* and the *Bathymodiolus* symbiont within that characteristic of *Thiomicrospira* (Kuenen and Tuovinen, 1981). Furthermore, the prokaryotic DNA comprised only about one third of the total *Bathymodiolus* gill DNA, while approximately 90% of the *Riftia* trophosome DNA was prokaryotic (Table I). Similarly, in a study of the 5S ribosomal RNA sequence in *Riftia* trophosome tissue, Stahl *et al.* (1984) found that approximately 75% of the total RNA was prokaryotic. These different proportions of eukaryotic *versus* prokaryotic material within the two hosts appears to be logical considering that the *Riftia* trophosome is believed to be specialized for harboring the symbionts whereas the mussel gill carries out other functions as well.

Chemoautotrophy, typical of sulfur oxidizing bacteria, was demonstrated by the effects of appropriate electron donors and oxygen on the incorporation of CO_2 into organic cell carbon. No CO_2 incorporation was found in the controls, *i.e.*, mantle tissue of *Bathymodiolus* and vestimentum tissue of *Riftia*. In the homogenized, symbiont-containing tissues the maximal CO_2 fixation rates were 44 and 13 nmol C mg^{-1} protein h^{-1} for *Bathymodiolus* and *Riftia*, respectively. Our assertion that these rates

measure principally autotrophic fixation is strengthened by the observation that they are of the same order of magnitude as the maximum ribulose-1,5-sebisphosphate carboxylase activities measured in these tissues by others (Cavanaugh *et al.*, 1981; Felbeck *et al.*, 1981) when based on the reasonable assumption that 1.0 g of tissue (wet wt) contains approximately 100 mg of protein. We presume that the variability in CO_2 incorporation rates measured in this study (see Figs. 2, 3) reflects different percentages of symbiont cells which were broken during tissue homogenization, contributing measurable amounts of protein but no CO_2 fixation.

The constancy of CO_2 incorporation per cell in the five preparations of *Bathymodiolus* gill homogenate can be taken as evidence for autotrophy in the mussel symbiont. Using a conversion factor of 1.2×10^{-13} gC μm^{-3} (Hagström, 1984), their average diameter of 0.6 μm translates into an approximate carbon content of 1.1×10^{-6} nmol per cell. This amount represents 60 to 140 times the hourly fixation rate per cell. These values imply relatively long doubling times, especially when compared to those of less than one hour as measured in free-living sulfur bacteria isolated from hydrothermal vents (Jannasch *et al.*, 1985).

The *Riftia* symbiont appears to be a short rod or sphere with diameters of approximately 3–5 μm (Cavanaugh *et al.*, 1981). The mussel gill symbiont, as noted above, is considerably smaller. These morphological differences between the two symbionts are paralleled by differences in their DNA base ratios, their primary use of different sulfur compounds as electron donors, their dissimilar temperature optima, and their response toward the presence of free oxygen.

Some of these differences may be reflected by the locations at which the two host animals are most commonly observed in the immediate vicinity of the vents. *Riftia* populations occur primarily near orifices of "warm" vents (Hessler and Smithey, 1983; Grassle, 1985; pers. obs.) where temperatures are in the range of 10 to 20°C and sulfide is prevalent (Edmond *et al.*, 1982; Jannasch and Mottl, 1985). Although mussels are often found intermingled with populations of tube worms, their bulk occurrence in dense beds immediately outside (with respect to vent orifices) of worm populations is in accordance with the psychrophilic characteristics of the *Bathymodiolus* symbiont.

It is of special interest that the *Bathymodiolus* symbiont appears to be the first sulfur-oxidizing prokaryote with truly psychrophilic growth characteristics. All free-living sulfur-oxidizing bacteria isolated so far from vent environments are mesophilic (Ruby and Jannasch, 1982; Jannasch *et al.*, 1985). The species designation of the mussel *B. thermophilus* (Kenk and Wilson, 1985) was based on its occurrence near warm vents and was published before the psychrophilic characteristics of their symbionts were found.

Additional evidence that the presence of sulfide within vent plumes is of more importance for the metabolism of *Riftia* than for that of *Bathymodiolus* is provided by enzymological data. Using a recently described assay (Powell and Somero, 1985) it was determined that sulfide oxidase activity in worm trophosome was 20 to 40 times higher than in the mussel gill tissue (Powell and Somero, in prep.). This is in accordance with the observation that no sulfide-binding protein, such as the one characteristic of *Riftia,* was found in *Bathymodiolus* preparations (J. J. Childress, pers. comm.).

The source of the thiosulfate used by the *Bathymodiolus* symbiont *in situ* is a point which is yet to be clarified. To our knowledge, no determinations of this anion in vent water were reported. The spontaneous oxidation of sulfide in seawater is known to proceed with a half-life of approximately one hour, with thiosulfate being a principal product (Almgren and Hagström, 1974). Thiosulfate can also be thermally produced from elemental sulfur (Belkin *et al.*, 1985). The fact that sulfide-stimulated CO_2 incorporation appears to commence eventually in the mussel symbiont (Fig. 1A, dashed line) suggests a thiosulfate-stimulation in disguise.

The *Bathymodiolus* symbiont appears to require free oxygen and incorporates CO_2 even at an atmospheric partial pressure of oxygen. The lack of a sustained aerobic CO_2 incorporation by the *Riftia* symbiont (Fig. 1B and unpub. obs.) cannot be attributed to an insufficient supply of sulfide because its replenishment did not prolong the period of linear incorporation. A similarly short-lived sulfide oxidation by trophosome preparations was observed by Fisher and Childress (1984).

A satisfactory explanation of the apparent anaerobic CO_2 incorporation by the *Riftia* symbiont (Fig. 4B) still requires a more detailed study. At this point, the data appear to indicate that free oxygen may be entirely replaced by hemoglobin-bound oxygen as proposed earlier (Arp and Childress, 1983). On the other hand, microaerophilism, a requirement for free oxygen at exceedingly low levels, is a common characteristic among the chemoautotrophic sulfur bacteria (Ruby and Jannasch, 1982; Jorgensen and Revsbech, 1983; Nelson and Jannasch, 1983).

Our recent observations at the Galapagos Rift vent site (May 1985) indicated profound environmental changes during the years elapsed since previous visits (January and December 1979). At the "Rose Garden" vent site, the extended "forests" of *Riftia* had all but disappeared and were replaced by extended mussel beds. *Riftia* occurred almost exclusively in two clumps each approximately 1 m in diameter. The maximum temperature in these limited worm populations was measured as 18°C (J. J. Childress, pers. comm.) and the concentration of H_2S was up to 300 μM (K. S. Johnson, pers. comm.). In contrast, the maximum temperature measured below mussels was 12°C and frequently dropped to ambient temperature of 2°C directly above the mussel beds (R. Lutz, pers. comm.). The sulfide concentration in the vicinity of mussels ranged from 0 to 100 μM (K. S. Johnson, pers. comm.). It appears that the remaining areas of strongest vent flow favored the maintenance of *Riftia* populations while the increasing dominance of *Bathymodiolus* may be a result of general decrease of the local hydrothermal vent activity. Our data indicate that these population changes reflect the specific metabolic properties of the two different chemoautotrophic symbionts.

Growing the symbionts in pure culture will still be necessary for their ultimate characterization. So far, isolation attempts from *Riftia* trophosome material resulted in organisms that did not resemble the symbionts in their diagnostic characteristics (Jannasch and Nelson, 1984). Studies, such as the present one, of the conditions under which prokaryotic preparations do sustain high rates of CO_2 incorporation, will aid further isolation attempts. Micro-oxic conditions and perhaps the involvement of oxygen-saturated *Riftia* blood possessing a sulfide-binding capacity (Arp and Childress, 1983) might have to be used. Since *Riftia* specimens are sensitive to a loss of pressure (Childress *et al.,* 1984), the possibility exists that its symbiont exhibits some barophilic characteristics. On the other hand, a broad pressure tolerance of prokaryotes, in general, as found so far in all sulfur-oxidizing bacteria isolated from the vents (Jannasch, 1984), might be offset by a pressure sensitivity of various host-symbiont interactions.

More optimism is justified in isolation experiments on the *Bathymodiolus* symbiont. Its sustained aerobic CO_2 incorporation at atmospheric pressure and its use of thiosulfate as an electron source render chances of its cultivation not more remote than that of similar symbionts now also found in shallow water clams (Cavanaugh, 1983; Felbeck *et al.,* 1984). To date, however, the latter have not yet been isolated either.

Acknowledgments

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